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**SECTION ONE –
 OVERVIEW OF TERRORISM**

Introduction to chemicals
 that have been studied
 following exposures of
 large populations

WHAT

**WHAT
 IS**

**WHAT
 IS
 TERRORISM?**

Terrorism first used to describe
 the new system of government
 adopted during the French
 Revolution
*Regime de
 la terreur*
 (Reign of
 Terror)




**CONTEMPORARY DEFINITION
 OF TERRORISM**



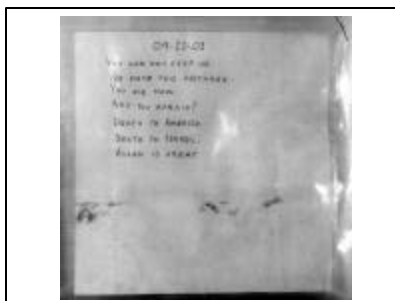

One month after September 11,
 2001 - a second
 wave of terrorism
 began

- BIOLOGICAL




This letter
 was postmarked
 September 16,
 2001 from Trenton,
 New Jersey

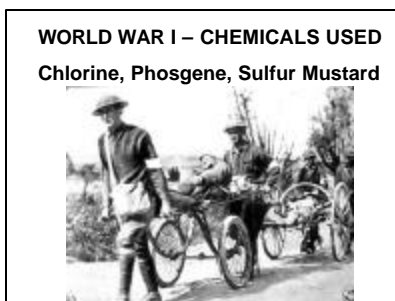




WHAT WOULD THE EMPHASIS IN INITIAL FUNDING FOR TERRORISM HAVE BEEN IF INSTEAD OF ANTHRAX – A TOXIC GAS HAD BEEN INTRODUCED INTO THE HART OFFICE BUILDING?

BIOLOGICAL vs. CHEMICAL TERRORISM		
Evaluation Parameter	Biological	Chemical
Detection	+ (?)	+
Acute Effect		+++
Latent Effect	+++	+ (?)
Low Cost Tech.		++
Efficient Weapon	+	++
Shock Factor	+++	+++

World War I demonstrated what effects chemicals could have as an agent of terror – most experts agree that chemical weapons had little tactical effect.



TYPES OF CHEMICAL AGENTS USED IN WW 1	
LUNG TOXICANTS	
Phosgene	<chem>Cl-C(=O)-Cl</chem>
Chlorine	<chem>Cl2</chem>

Germans used Cl_2 first at Ypres on 22 April 1915 – French and British later began use



French gas attack on German lines, Belgium, 1916



Approximately 150-169 tons of Cl_2 was released along a 7 km front with the gas engulfing the Franco-Algerian soldiers – Since the attack was unexpected about 3,000 soldiers died

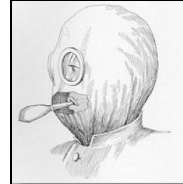


31 May 1915, Germany discharged 220 tons of phosgene along a 12 km width against Russian troops – the number of deaths are unknown

Most deaths occurred early in the war prior to wide-spread use of gas masks or other crude protection



French soldier In a trench wearing a face mask

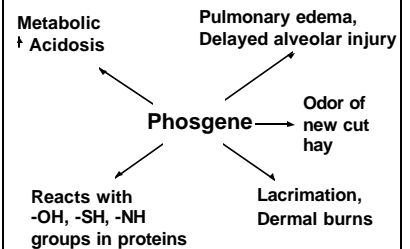
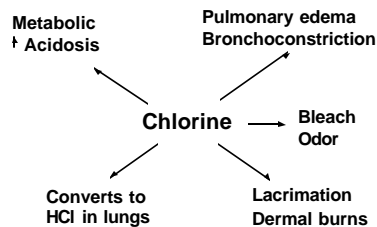


British gas helmet, 1916

British soldier wearing a gas mask



American Marines wearing respirators



Later, chemicals were placed in munitions, the most notable being mustard gas



Livens Projectors for delivering gas shells



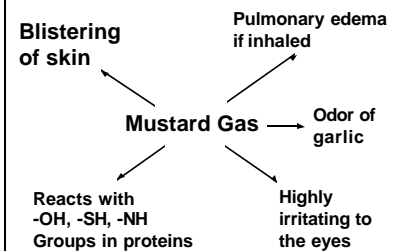
French poison gas canister

Mustard gas was first used by Germany in the trenches near Ypres, Belgium in September, 1917 – led to the term “Yperite”



TYPES OF CHEMICAL WEAPONS USED IN WW I

VESICANTS
HD (Mustard)
bis-2-Chloroethylsulfide



British soldiers blinded by mustard gas, circa September 1917



John Singer Sargent's painting – “Gassed” Hangs in Imperial War Museum in London



HUMAN EXPOSURES - SULFUR MUSTARD (HD, HT) -

Symptoms:

- Fluid filled blisters
- Second or third degree-like burns
- Eye burns & blurred vision
- Hemorrhagic lesions of tracheae & alveolar edema
- Non-fatal incapacitation



An Iranian soldier exposed to mustard gas



SUMMARY OF LESSONS LEARNED FROM WW1 CHEMICALS

- Deaths and incapacitation can be wide-spread
- Terror was prevalent
- Crude masks were effective in preventing injury
- Delivery was unpredictable

SECTION TWO – POTENTIAL TERRORIST CHEMICALS

- Previous events

THE TYLENOL MURDERS

29 Sept – 1 Oct, 1982



7 People died in the Chicago area from cyanide put in Tylenol capsules

“ANARCHIST” PLEADS GUILTY - Cyanide Hidden in Chicago’s Transit System – Nov 22, 2002

Joseph Konopka, 25 who called himself, “Dr. Chaos” possessed and stored sodium cyanide and sodium carbonate – he had been previously arrested for sabotage



CYANIDE THREAT IN NEW ZEALAND

Aug 19, 2003 – Counter-terrorism Chief Jon White investigating letters threaten water supplies and use of gas in cinemas



CYANIDE PLOT ON LONDON UNDERGROUND TUBE

Nov 17, 2002 – MI5 agents foil plot of North Africans loosely associated with al Qaeda to release cyanide in the Tube



SHIPMENT OF CYANIDE STOLEN IN MEXICO

May 16, 2002 – Mexican police find a stolen truck carrying 10 tons of sodium cyanide



CYANIDE BUST IN ROME

Feb 20, 2002 – 4 Moroccans were arrested south of Rome in possession of a large amount of cyanide



CYANIDES

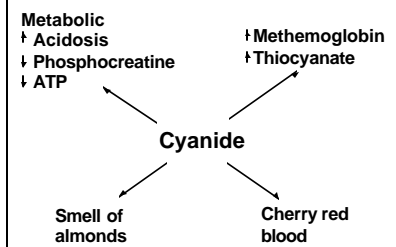
Hydrogen cyanide



Cyanogen chloride



Sodium cyanide



CYANIDE ANTIDOTE KIT

1. Inhale amyl nitrite from ampoule
2. IV sodium nitrite infusion
3. IV sodium thiosulfate



POTENTIAL TERRORIST CHEMICALS

- Previous events
- Industrial chemicals

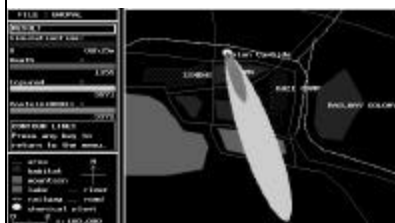
EXAMPLE OF TOXIC CHEMICAL EXPOSURE - METHYLISOCYANATE -

Bhopal, India – December 3, 1984

Just after midnight the Union Carbide plant accidentally released 40 metric tons of methyl isocyanate into the atmosphere



The plumb of toxic gas spread over adjacent "fence-line" communities



ESTIMATES OF 2,000 CASUALTIES



ESTIMATES OF 100,000 INJURIES

Acute exposures involved eye and respiratory symptoms



Animals were also affected

POTENTIAL TERRORIST CHEMICALS

- Previous events
- Industrial chemicals
- Military chemical weapons

MILITARY CHEMICAL WEAPONS

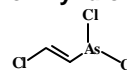
May provide clues as to the type and physiological actions expected from potential chemical terrorism agents

POST – WW I CHEMICALS WEAPONS

- Blood agents – cyanides
- Blister agents – Lewisite
- Eye and vomiting (choking) agents – CN, DM
- Hallucinogenic agents – BZ
- Nerve gases – sarin, VX
- Anesthetic agents - fentanyl

TYPES OF CHEMICAL WARFARE AGENTS

VESICANTS (blisters)
L (Lewisite)
2-Chlorovinyl dichloroarsine



Arsenic toxidromes occur in a latent fashion

Absorbed through clothing, skin, inhalation

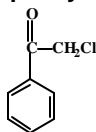
Lewisite

Smell of geraniums

Contact with skin produces delayed blisters

TYPES OF CHEMICAL WARFARE AGENTS

TEAR GASES (CN)
2-Chloro-1-phenylethanone (mace)



Rhinorrhea, sneezing, eye pain, skin irritation

Effects last for 30 min – longer for skin effects

Chloroacetophenone (CN)

Odor of apple blossoms

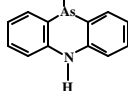
Lacrimator due to interaction with sulfhydryl groups

TYPES OF CHEMICAL WARFARE AGENTS

VOMITING GAS

DM (Adamsite)

10-Chloro-5,10-dihydrophen-arsazine



Sickness similar to "seasickness"

Effects begin about 6 min after exposure

Diphenylaminochlorarsine (DM)

Arsenic toxidromes

Sever pain in the nose and chest followed by vomiting

COMBINATION USE OF CN AND DM

DM requires about 6 min to start its action

CN has an immediate effect on the eyes

TERRORISM REPORT USING DM (ADAMSITE)

Brussels, Belgium – June 4, 2003

Brownish-yellow powder mailed in envelopes to Saudi Arabian Embassy, 3 ministries, airport, and port authority

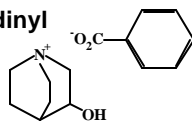
2 postal workers taken to hospital

TYPES OF CHEMICAL WARFARE AGENTS

INCAPACITATION

BZ

3-Quinuclidinyl benzilate



0.5 Hrs after exposure

↓ HR & respiration
dizziness
dry mouth

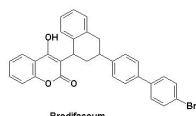
3-5 Hrs after exposure
- incapacitation

BZ

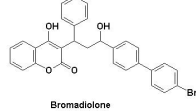
"super" hallucinogen
6-10 Hrs after exposure

Antagonizes muscarinic receptors

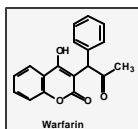
"SUPER WARFARINS"



Brodifacoum



Bromadiolone



Warfarin

Epistaxis, gingival bleeding, hematemesis, hematuria, melena, hemoptysis

Unexplained bleeding

"Super Warfarins"

Detection in biological or environmental samples

Prolonged PT

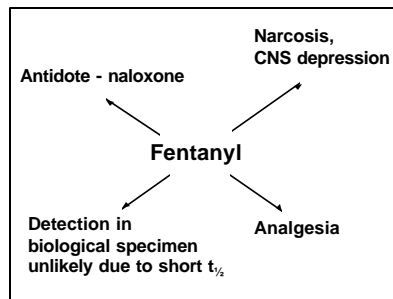
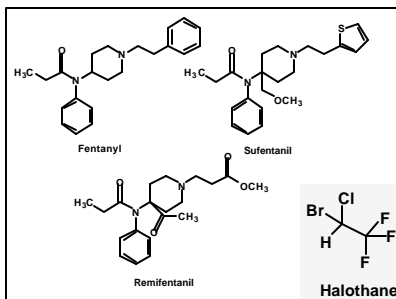
RUSSIAN "KNOCK-OUT GAS"

116 Persons died in a Moscow theater in the aftermath of Chechen terrorist event



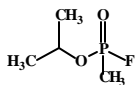
CONTENT OF RUSSIAN GAS

- Not known with certainty – fentanyl & halothane suspected
- First use of a mid-spectrum agent – falls between the classical definition of a chemical or biological weapon



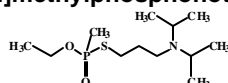
TYPES OF CHEMICAL WARFARE AGENTS

NERVE AGENTS GB (Sarin) Isopropylmethylphosphonofluoridate

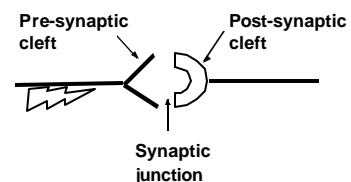


TYPES OF CHEMICAL WARFARE AGENTS

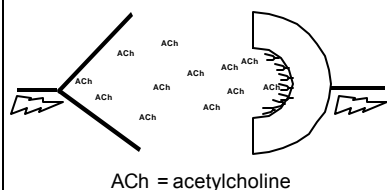
NERVE AGENTS (cont'd) VX O-Ethyl-S-[2-diisopropylamino)ethyl]methylphosphonothiolate



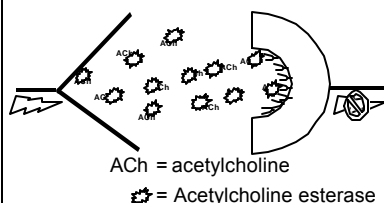
CHOLINERGIC SYNAPSE



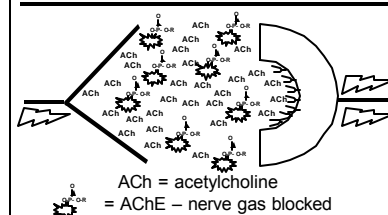
CHOLINERGIC NERVE TRANSMISSION

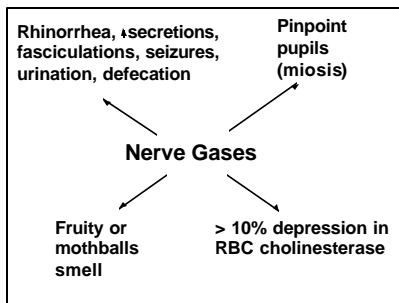


TERMINATION OF CHOLINERGIC TRANSMISSION



NERVE GAS BLOCKADE OF ACETYLCHOLINESTERASE





HUMAN EXPOSURES - SARIN (GB) -

Aum Shinrikyo cult performed two acts of terrorism in Japan

- Matsumoto – June 27, 1994
- Tokyo – March 20, 1995



SARIN RELEASE

- Matsumoto - large fan from the back of a truck
- Tokyo - plastic bags opened under subway seats

HUMAN EXPOSURES - SARIN (GB) -

Matsumoto - city of 200,000

- At 2100 hrs people began to sneeze or have rhinorrhea
- First ambulance call - 2309 hrs

HUMAN EXPOSURES - SARIN (GB) -

Matsumoto - first call

- Man's wife had loss consciousness & dog died in garden.
- Man was nauseated with clouded vision

HUMAN EXPOSURES - SARIN (GB) -

Matsumoto - 0010 Hrs

- Situation judged to be a mass disaster
- Police & rescue personnel began search of area

HUMAN EXPOSURES - SARIN (GB) -

Matsumoto - search & rescue

- 3 Found dead in rooms
- 4 Died shortly after evacuation
- 53 Admitted to hospital
- 253 Seen as out-patients

HUMAN EXPOSURES - SARIN (GB) -
Matsumoto - two exposure peaks
<ul style="list-style-type: none"> Immediately following release Approximately 8 hrs. later

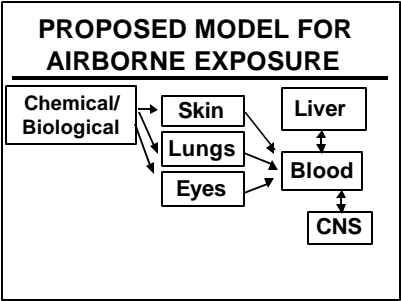
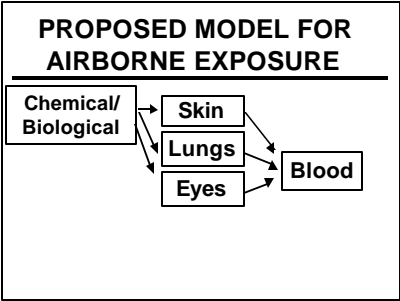
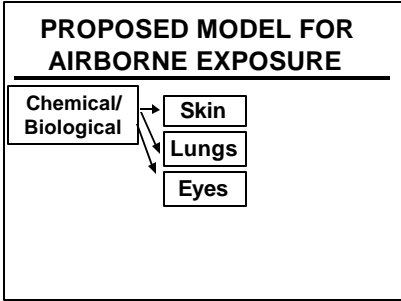
HUMAN EXPOSURES - SARIN (GB) -
Tokyo - numbers involved
<ul style="list-style-type: none"> 5,510 sought medical care at 200 hospitals & clinics Approximately 1,300 hospitalized 12 deaths

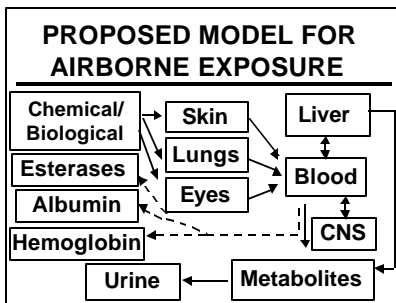
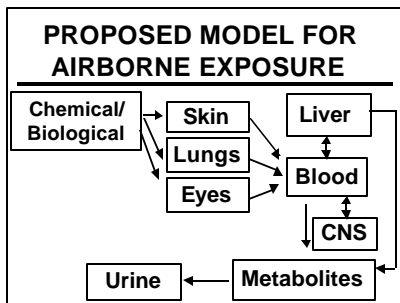
TOKYO SUBWAY CASE REPORT Lancet 347:13-43, 1996
<ul style="list-style-type: none"> 35 yr old male 7 min after exposure has <ul style="list-style-type: none"> tonic-clonic convulsions episodes of dyspnea comatose & cyanotic upon ED presentation pinpoint pupils profuse sweating, nasal secretions & vomiting

TOKYO SUBWAY CASE REPORT (continued)
<ul style="list-style-type: none"> atropine & pralidoxime given regained consciousness 8 hrs later disoriented & impaired short-term memory EEG changes for 3 mos RBC cholinesterase depressed for 3 mos

SECTION THREE – LABORATORY DETECTION OF EXPOSURE
<p>Concepts of body fluid testing</p>

PROPOSED MODEL FOR AIRBORNE EXPOSURE
<div>Chemical/ Biological</div>





LINES OF DEFENSE TO AIRBORNE TOXICANTS

- **SKIN** - most substances do not penetrate
- **LUNGS** - first organ of metabolism
- **EYES** - limited absorption

LINES OF DEFENSE TO AIRBORNE TOXICANTS (continued)

- **LIVER METABOLISM**
 - saturation
 - non-toxic & toxic metabolites
- **BLOOD ESTERASES** - may either bind (stoichiometric) or hydrolyze (catalytic)



LABORATORY TESTING

Exposure Testing

- Has exposure occurred?

BIO-MONITORING OF NERVE AGENT EXPOSURE USING CHOLINESTERASE

Principle - exposure to nerve agents will produce a depression in cholinesterases (ChE)

BIO-MONITORING OF NERVE AGENT EXPOSURE USING CHOLINESTERASE – cont'd

Acetylcholine (ACh) is the neuro- transmitter for the cholinergic nervous system

Acetylcholinesterase (AChE) hydrolyzes ACh

**BIO-MONITORING OF NERVE
AGENT EXPOSURE USING
CHOLINESTERASE – cont'd**

Two cholinesterase forms:

- Plasma or serum cholinesterase (pseudocholinesterase or butyrylcholinesterase)
- RBC cholinesterase

**BIO-MONITORING OF NERVE
AGENT EXPOSURE USING
CHOLINESTERASE – cont'd**

RBC-ChE uses ACh as its substrate

Bu-ChE uses butyrylcholine as its substrate

**BIO-MONITORING OF NERVE
AGENT EXPOSURE USING
CHOLINESTERASE – cont'd**

Bu-ChE is polymorphic

RBC-ChE is not polymorphic

Both have ~90 day turnover

**BIO-MONITORING OF NERVE
AGENT EXPOSURE USING
CHOLINESTERASE – cont'd**

Method is non-specific since other organophosphates, eg. pesticides will also cause ChE depression

**BIO-MONITORING OF NERVE
AGENT EXPOSURE USING
CHOLINESTERASE – cont'd**

RBC-ChE or Bu-ChE level must be < 85% of the normal or baseline level to indicate exposure – Tox Lett 124:87, 2002

**BIO-MONITORING OF NERVE
AGENT EXPOSURE USING
CHOLINESTERASE – cont'd**

- Requires knowing individual's enzymatic baseline or normal due to wide inter-individual variability

**BIO-MONITORING OF NERVE
AGENT EXPOSURE USING
CHOLINESTERASE – cont'd**

- Not adequate to compare to a population based normal (CV ~ 10-15%)

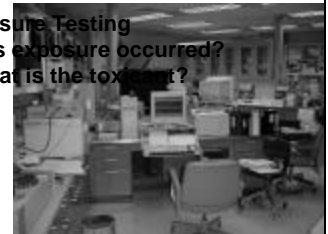
**BIO-MONITORING OF NERVE
AGENT EXPOSURE USING
CHOLINESTERASE – cont'd**

- Not a good marker of mild exposure to nerve agents

LABORATORY TESTING

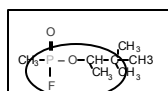
Exposure Testing

- Has exposure occurred?
- What is the toxin?

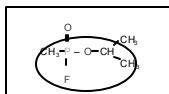


IMMUNOASSAYS TO DETECT NERVE AGENTS

Soman (GD)



Sarin (GB)



Monoclonal prepared using a Soman analogue-BSA conjugate. Conc. giving 50% inhibition Soman 2.5×10^{-7} M/L, Sarin 8×10^{-8} M/L

Immuno Letters 31:131-136:1992
J Appl. Tox. 21:523-526:2001

LOD - IMMUNOASSAYS vs CHOLINESTERASE

Sarin ^{1,2}	140 ng/mL
Soman ^{2,3}	90 ng/mL
VX ⁴	<0.2 ng/mL
HD ⁵	~800 ng/mL
ChE inhibition	<10 pg/mL

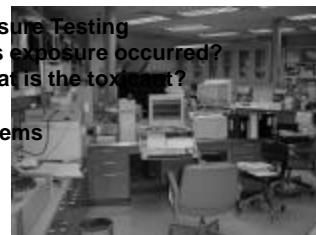
1. Arch Toxicol 69:644-46:1996 4. Arch Toxicol 67:6671:1993
2. J Appl Toxicol 21:523-6:2001 5. Anal Biochem 257:12-6:1998
3. Arch Toxicol 64:580-85:1990

LABORATORY TESTING

Exposure Testing

- Has exposure occurred?
- What is the toxin agent?

Problems



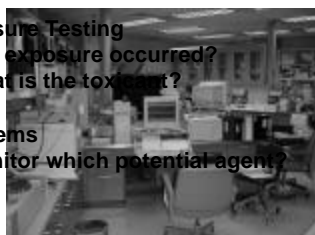
LABORATORY TESTING

Exposure Testing

- Has exposure occurred?
- What is the toxin agent?

Problems

- Monitor which potential agent?



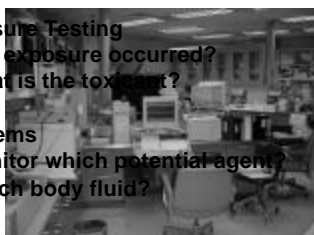
LABORATORY TESTING

Exposure Testing

- Has exposure occurred?
- What is the toxin agent?

Problems

- Monitor which potential agent?
- Which body fluid?



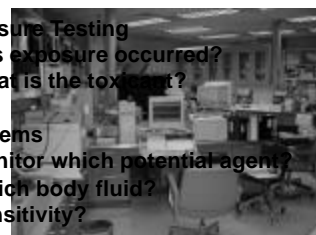
LABORATORY TESTING

Exposure Testing

- Has exposure occurred?
- What is the toxin agent?

Problems

- Monitor which potential agent?
- Which body fluid?
- Sensitivity?



ADVANTAGES OF URINE IN TESTING FOR EXPOSURE



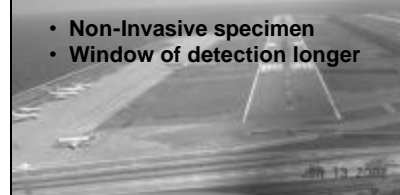
ADVANTAGES OF URINE IN TESTING FOR EXPOSURE

- Non-Invasive specimen



ADVANTAGES OF URINE IN TESTING FOR EXPOSURE

- Non-Invasive specimen
- Window of detection longer



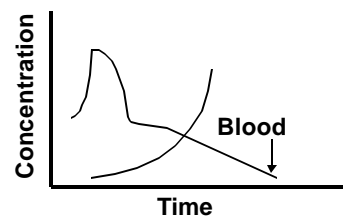
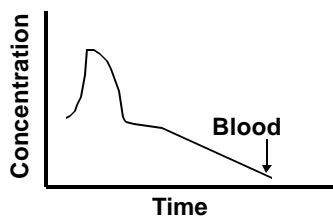
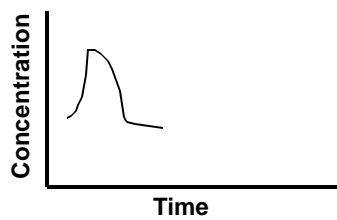
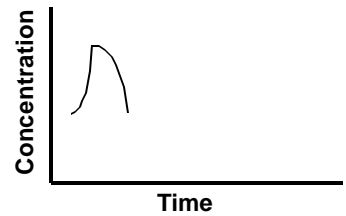
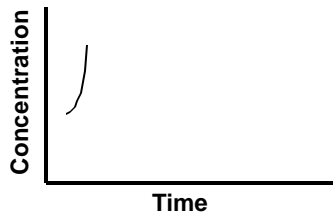
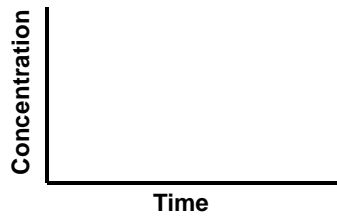
ADVANTAGES OF URINE IN TESTING FOR EXPOSURE

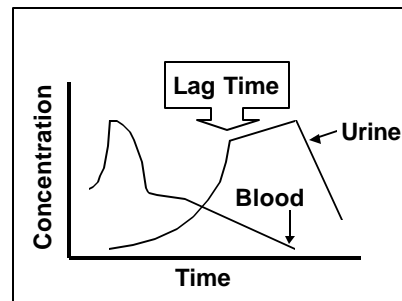
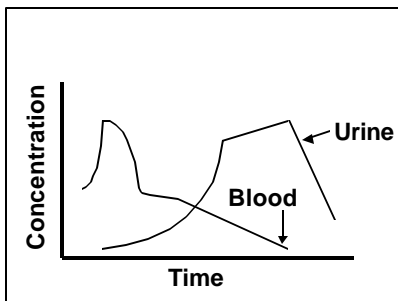
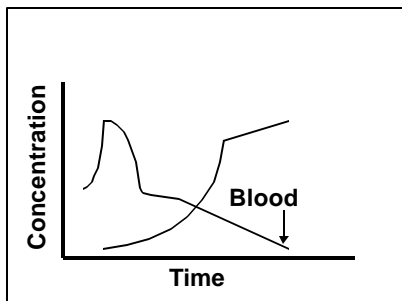
- Non-Invasive specimen
- Window of detection longer
- Analyte of interest in greater concentration

DISADVANTAGES OF URINE IN TESTING FOR EXPOSURE

DISADVANTAGES OF URINE IN TESTING FOR EXPOSURE

- Lag time for elimination





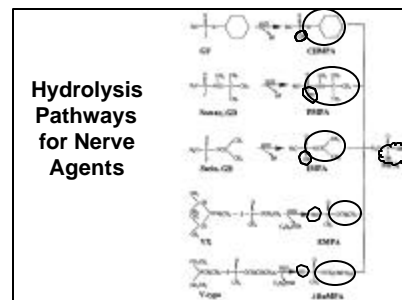
DISADVANTAGES OF URINE IN TESTING FOR EXPOSURE

- Lag time for elimination
- Metabolites must be analyzed

ASSAY FOR PARENT AGENT OR METABOLITE(S)

Consider chemical warfare agents

- Nerve gases
- HD (sulfur mustard)



SULFUR MUSTARD METABOLISM

Parent compound does not appear in urine

–Major metabolite, thiodiglycol

$$\begin{array}{ccc}
 \text{S} & \xrightarrow{\quad} & \text{S} \\
 | & & | \\
 \text{CH}_2\text{CH}_2\text{Cl} & & \text{CH}_2\text{CH}_2\text{OH} \\
 | & & | \\
 \text{CH}_2\text{CH}_2\text{Cl} & & \text{CH}_2\text{CH}_2\text{OH}
 \end{array}$$

Sulfur mustard (HD) Thiodiglycol

DISADVANTAGES OF URINE IN TESTING FOR EXPOSURE

- Lag time for elimination
- Metabolites must be analyzed
- Analyte may be endogenous

SULFUR MUSTARD METABOLISM – cont'd

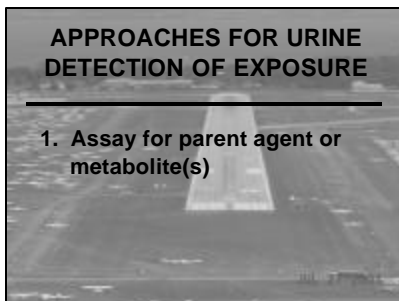
Thiodiglycol is an endogenous constituent of:

- Normal urine although at very low levels (< 1 ng/mL)
- Blood ~16 ng/mL
- Ground water – 4-16 ng/mL

APPROACHES FOR URINE DETECTION OF EXPOSURE

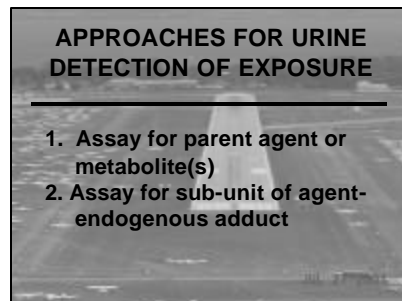


APPROACHES FOR URINE DETECTION OF EXPOSURE



1. Assay for parent agent or metabolite(s)

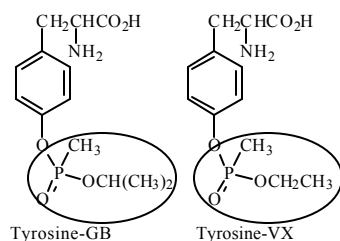
APPROACHES FOR URINE DETECTION OF EXPOSURE



1. Assay for parent agent or metabolite(s)
2. Assay for sub-unit of agent-endogenous adduct

ASSAY FOR SUB-UNIT OR ENDOGENOUS ADDUCTS

- Interaction of nerve gas with amino acids in
 - Albumin
 - Hemoglobin



CONCLUSIONS

- Lessons learned from past

LESSONS LEARNED FROM PREVIOUS TERRORIST EVENTS

1. Unsophisticated group can get materials for mass destruction
2. Atmospheric dissemination can be readily accomplished
3. Human toxicity can be profound

LESSONS LEARNED FROM PREVIOUS TERRORIST EVENTS (continued)

4. Toxic agent may not be readily recognized
5. Medical facilities can be overwhelmed
6. Medical & rescue workers are at risk

CONCLUSIONS – cont'd

- Lessons learned from past
- Urine and blood can augment information concerning toxic exposure(s)

CONCLUSIONS – cont'd

- Urine or blood can augment information concerning toxic exposure(s)
- ChE testing may indicate OP exposure

CONCLUSIONS – cont'd

- Urine or blood can augment information concerning toxic exposure(s)
- ChE testing may indicate OP exposure
- Assay for the parent agent or metabolite is optimum

